

Structure search

Venci 10/715,329

07/02/2005

=> d his ful

FILE 'HCAPLUS' ENTERED AT 10:20:05 ON 07 FEB 2005

E FALCK JOHN R/AU
L1 160 SEA ABB=ON "FALCK JOHN R"/AU
E ZHAO YINGMING/AU
L2 48 SEA ABB=ON ("ZHAO YINGMIN"/AU OR "ZHAO YINGMING"/AU)
L3 1 SEA ABB=ON L1 AND L2
D TI
SELECT RN L3 1-1

FILE 'REGISTRY' ENTERED AT 10:21:44 ON 07 FEB 2005

L4 STR
L5 0 SEA SSS SAM L4
L6 0 SEA SSS FUL L4
L7 STR L4
L8 0 SEA SSS SAM L7
L9 0 SEA SSS FUL L7
L10 STR L7
L11 0 SEA SSS SAM L10
L12 STR L10
L13 0 SEA SSS SAM L12
L14 0 SEA SSS FUL L12

~~FILE 'HCAPLUS' ENTERED AT 11:30:23 ON 07 FEB 2005~~

~~E MORITANI TOHEI/AU
L15 35 SEA ABB=ON "MORITANI TOHEI"/AU
E ALVAREZ LORENZO CARMEN/AU
L16 50 SEA ABB=ON ("ALVAREZ LORENZO C"/AU OR "ALVAREZ LORENZO
CARMEN"/AU)
L17 2 SEA ABB=ON L15 AND L16
SELECT RN L17 1
E US2003-715329/AP~~

FILE 'REGISTRY' ENTERED AT 11:42:34 ON 07 FEB 2005

L18 STR L12
L19 0 SEA SSS SAM L12
L20 0 SEA SSS FUL L12

FILE 'MARPAT' ENTERED AT 11:43:16 ON 07 FEB 2005

L21 0 SEA SSS SAM L12
L22 0 SEA SSS FUL L12

FILE 'BEILSTEIN' ENTERED AT 11:43:41 ON 07 FEB 2005

L23 0 SEA ABB=ON L13

FILE 'REGISTRY' ENTERED AT 11:43:54 ON 07 FEB 2005

L24 STR L13
L25 0 SEA SSS SAM L24
L26 0 SEA SSS FUL L24

FILE 'MARPAT' ENTERED AT 11:45:00 ON 07 FEB 2005

L27 0 SEA SSS SAM L24
L28 STR L24
L29 0 SEA SSS SAM L28
L30 0 SEA SSS FUL L28
L31 STR L28

see attached d gne stat for Marpat

FILE 'BEILSTEIN' ENTERED AT 12:01:30 ON 07 FEB 2005

L32 0 SEA SSS SAM L28
L33 0 SEA SSS FUL L28

see attached & gne stat for Beilstein

FILE 'REGISTRY' ENTERED AT 12:02:56 ON 07 FEB 2005

L34 0 SEA SSS SAM L28
L35 0 SEA SSS FUL L28

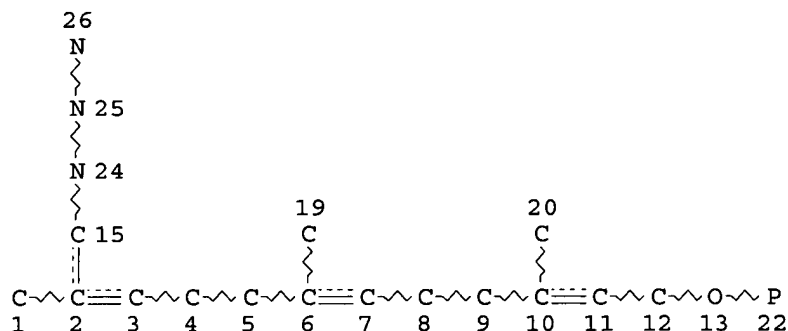
*see attached & gne stat
for CA Plus*

David,
As I mentioned in a phone message, I've
consulted with Paul Schulwitz, and
~~I haven't~~ haven't come up with any ideas
for these structures in CA Plus, Mapset, or
Beilstein. If you have any further ideas,
please let me know.

Mary Jane Ruhl
X22524

P.S. I also checked with Lora Burgess
of STN, and she could not think of
any other approach.

```
=> d que stat 135
L28          STR
```



NODE ATTRIBUTES:
 DEFAULT MLEVEL IS ATOM
 DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS 20

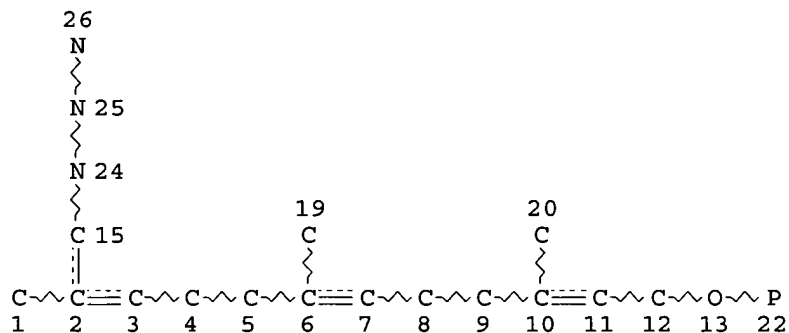
STEREO ATTRIBUTES: NONE
L35 0 SEA FILE=REGISTRY SSS FUL L28

```
100.0% PROCESSED      163 ITERATIONS
SEARCH TIME: 00.00.01
```

0 ANSWERS

White in CA Plus

```
=> d que stat 133
L28          STR
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NODE ATTRIBUTES:
 DEFAULT MLEVEL IS ATOM
 DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS 20

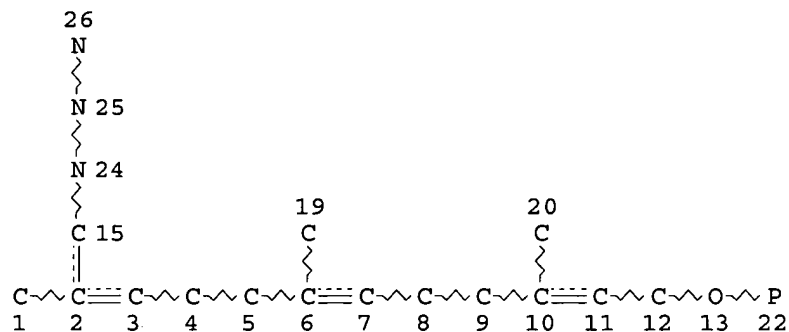
STEREO ATTRIBUTES: NONE
L33 0 SEA FILE=BEILSTEIN SSS FUL L28

100.0% PROCESSED 78 ITERATIONS

0 ANSWERS

chutz in Bechtstein

```
=> d que stat l31
L31          STR
```



NODE ATTRIBUTES:
 DEFAULT MLEVEL IS ATOM
 DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS 20

STEREO ATTRIBUTES: NONE

Shifts in Mergat

=> d his ful

FILE 'HCAPLUS' ENTERED AT 17:58:58 ON 07 FEB 2005
L1 2178 SEA ABB=ON ?FARNESYLAT? OR ?ISOPRENYLAT?
L2 2 SEA ABB=ON L1 AND (?AZID?(3A)?FARNESYL? OR TAG?(2W)?AZID?(W)?S
UBSTRAT? OR TAS OR ?AZID?(3A)TAG?)
L3 919 SEA ABB=ON L1 AND ((?PHOSPHIN?(3A)?CAPTUR?(W)(?REAGENT? OR
?REACT?)) OR ?SUBSTRAT? OR ?SOLID?(W)(?PHASE? OR ?SUPPORT?) OR
?BEAD? OR ?RESIN? OR ?MEMBRAN? OR ?PARTICL? OR ?PARTICUL? OR
?TRIPHENYLPHOSPHIN? OR ?IMINOPHOSPHORAN? OR ?STAUDINGER?)
L4 15 SEA ABB=ON L1 AND (?METABOL?(W)?INCORPOR? OR ?CELL?(W)(?UPTAK?
OR ?PERMEAB? OR ?INCORPORAT?) OR IN(W)?VIVO?(W)?LABEL?)
L5 10 SEA ABB=ON L3 AND L4
L6 11 SEA ABB=ON L2 OR L5

FILE 'MEDLINE, BIOSIS, EMBASE, JAPIO, JICST-EPLUS, COMPENDEX' ENTERED AT
18:08:04 ON 07 FEB 2005
L7 36 SEA ABB=ON L6
L8 15 DUP REMOV L7 (21 DUPLICATES REMOVED)

```
=> d que stat 16
L1      2178 SEA FILE=HCAPLUS ABB=ON  ?FARNESYLAT? OR ?ISOPRENYLAT?
L2      2 SEA FILE=HCAPLUS ABB=ON  L1 AND (?AZID?(3A)?FARNESYL? OR
      TAG?(2W)?AZID?(W)?SUBSTRAT? OR TAS OR ?AZID?(3A)TAG?)
L3      919 SEA FILE=HCAPLUS ABB=ON  L1 AND ((?PHOSPHIN?(3A)?CAPTUR?(W)(?RE
      AGENT? OR ?REACT?)) OR ?SUBSTRAT? OR ?SOLID?(W)(?PHASE? OR
      ?SUPPORT?) OR ?BEAD? OR ?RESIN? OR ?MEMBRAN? OR ?PARTICL? OR
      ?PARTICUL? OR ?TRIPHENYLPHOSPHIN? OR ?IMINOPHOSPHORAN? OR
      ?STAUDINGER?)
L4      15 SEA FILE=HCAPLUS ABB=ON  L1 AND (?METABOL?(W)?INCORPOR? OR
      ?CELL?(W)(?UPTAK? OR ?PERMEAB? OR ?INCORPORAT?) OR IN(W)?VIVO?(
      W)?LABEL?)
L5      10 SEA FILE=HCAPLUS ABB=ON  L3 AND L4
L6      11 SEA FILE=HCAPLUS ABB=ON  L2 OR L5
=> d ibib abs 16 1-11
```

L6 ANSWER 1 OF 11 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2004:743418 HCAPLUS

DOCUMENT NUMBER: 141:362701

TITLE: A tagging-via-**substrate** technology for
detection and proteomics of **farnesylated**
proteins

AUTHOR(S): Kho, Yoonjung; Kim, Sung Chan; Jiang, Chen; Barma,
Deb; Kwon, Sung Won; Cheng, Jinke; Jaunbergs, Janis;
Weinbaum, Carolyn; Tamanoi, Fuyuhiko; Falck, John;
Zhao, Yingming

CORPORATE SOURCE: Department of Biochemistry, University of Texas
Southwestern Medical Center, Dallas, TX, 75390-9038,
USA

SOURCE: Proceedings of the National Academy of Sciences of the
United States of America (2004), 101(34), 12479-12484
CODEN: PNASA6; ISSN: 0027-8424

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A recently developed proteomics strategy, designated tagging-via-**substrate** (TAS) approach, is described for the detection and proteomic anal. of **farnesylated** proteins. TAS technol. involves **metabolic incorporation** of a synthetic **azido-farnesyl** analog and chemoselective derivatization of **azido-farnesyl**-modified proteins by an elegant version of **Staudinger** reaction, pioneered by the Bertozzi group, using a biotinylated **phosphine capture reagent**. The resulting protein conjugates can be specifically detected and/or affinity-purified by streptavidin-linked horseradish peroxidase or agarose **beads**, resp. Thus, the technol. enables global profiling of **farnesylated** proteins by enriching **farnesylated** proteins and reducing the complexity of **farnesylation** subproteome. **Azido-farnesylated** proteins maintain the properties of protein **farnesylation**, including promoting **membrane** association, Ras-dependent mitogen-activated protein kinase k activation, and inhibition of lovastatin-induced apoptosis. A proteomic anal. of **farnesylated** proteins by TAS technol. revealed 18 **farnesylated** proteins, including those with potentially novel **farnesylation** motifs, suggesting that future use of this method is likely to yield novel insight into protein **farnesylation**. TAS technol. can be extended to other posttranslational modifications, such as geranylgeranylation and myristoylation, thus providing powerful tools for detection, quantification, and proteomic anal. of posttranslationally

modified proteins.

REFERENCE COUNT: 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 11 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2004:446416 HCAPLUS

DOCUMENT NUMBER: 141:687

TITLE: Apoptotic and Cytostatic Farnesyltransferase Inhibitors Have Distinct Pharmacology and Efficacy Profiles in Tumor Models

AUTHOR(S): Manne, Veeraswamy; Lee, Francis Y. F.; Bol, David K.; Gullo-Brown, Johnni; Fairchild, Craig R.; Lombardo, Louis J.; Smykla, Richard A.; Vite, Gregory D.; Wen, Mei-Li D.; Yu, Chiang; Wong, Tai Wai; Hunt, John T.

CORPORATE SOURCE: Oncology Drug Discovery and Discovery Chemistry, Bristol-Myers Squibb Company Pharmaceutical Research Institute, Princeton, NJ, USA

SOURCE: Cancer Research (2004), 64(11), 3974-3980

CODEN: CNREA8; ISSN: 0008-5472

PUBLISHER: American Association for Cancer Research

DOCUMENT TYPE: Journal

LANGUAGE: English

AB BMS-214662 and BMS-225975 are tetrahydrobenzodiazepine-based farnesyltransferase inhibitors (FTIs) that have nearly identical structures and very similar pharmacol. profiles associated with farnesyltransferase (FT) inhibition. Despite their similar activity against FT in vitro and in cells, these compds. differ dramatically in their apoptotic potency and tumor-regressing activity in vivo. BMS-214662 is the most potent apoptotic FTI known and exhibits curative responses in mice bearing a variety of staged human tumor xenografts such as HCT-116 human colon tumor. By contrast, BMS-225975 does not cause tumor regression and at best causes partial tumor growth inhibition in staged HCT-116 human colon tumor xenografts. Lack of tumor regression activity in BMS-225975 was attributable to its relatively weak apoptotic potency, not to poor **cell permeability** or pharmacokinetics. Both compds. were equally effective in inhibiting Ras processing and causing accumulation of a variety of **nonfarnesylated substrates** of FT in HCT-116 cells. Because BMS-225975 has poor apoptotic activity compared with BMS-214662 but inhibits FT to the same extent as BMS-214662, it is very unlikely that FT inhibition alone can account for the apoptotic potency of BMS-214662. Clearly distinct patterns of sensitivities in a cell line panel were obtained for the apoptotic FTI BMS-214662 and the cytostatic FTI BMS-225975. Activation of the c-Jun-NH2-terminal kinase pathway was readily observed with BMS-214662 but not with BMS-225975. We developed a highly sensitive San-1 murine xenograft tumor model that is **particularly** useful for evaluating the in vivo activity of cytostatic FTIs such as BMS-225975.

REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 11 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2002:453795 HCAPLUS

DOCUMENT NUMBER: 137:165381

TITLE: Photoaffinity Analogues of Farnesyl Pyrophosphate Transferable by Protein Farnesyl Transferase

AUTHOR(S): Chehade, Kareem A. H.; Kiegiel, Katarzyna; Isaacs, Richard J.; Pickett, Jennifer S.; Bowers, Katherine E.; Fierke, Carol A.; Andres, Douglas A.; Spielmann, H. Peter

CORPORATE SOURCE: Department of Molecular and Cellular Biochemistry

Department of Chemistry Kentucky Center for Structural
Biology, University of Kentucky, Lexington, KY,
40536-0084, USA

SOURCE: Journal of the American Chemical Society (2002),
124(28), 8206-8219
CODEN: JACSAT; ISSN: 0002-7863

PUBLISHER: American Chemical Society

DOCUMENT TYPE: Journal

LANGUAGE: English

OTHER SOURCE(S): CASREACT 137:165381

AB **Farnesylation** is a posttranslational lipid modification in which
a 15-carbon farnesyl isoprenoid is linked via a thioether bond to specific
cysteine residues of proteins in a reaction catalyzed by protein
farnesyltransferase (FTase). We synthesized analogs (3-6) of farnesyl
pyrophosphate (FPP) to probe the range of modifications possible to the
FPP skeleton which allow for efficient transfer by FTase. Photoaffinity
analogs of FPP (5, 6) were prepared by substituting perfluorophenyl azide
functional groups for the ω -terminal isoprene of FPP. Substituted
anilines replace the ω -terminal isoprene in analogs 3 and 4.
Compds. 3-5 were prepared by reductive amination of the appropriate anilines
with 8-oxo-geranyl acetate, followed by ester hydrolysis, chlorination,
and pyrophosphorylation. Addnl. substitution of three methylenes for the
 β -isoprene of FPP gave photoprobe 6 in nine steps. Preparation of the
analogs required TiCl_4 -mediated imine formation prior to $\text{NaBH}(\text{OAc})_3$ reduction
for anilines with a $\text{pK}_a < 1$. The azide moiety was not affected by Ph_3PCl_2
conversion of allylic alcs. 13-16 into corresponding chlorides 17-20.
Analog 3-6 are efficiently transferred to target N-dansyl-GCVLS peptide
substrate by mammalian FTase. Comparison of analog structures and
kinetics of transfer to those of FPP reveals that ring fluorination and
para substituents have little effect on the affinity of the analog
pyrophosphate for FTase and its transfer efficiency. These results are
also supported with models of the analog binding modes in the active site
of FTase. The transferable azide photoprobe 5 photoinactivates FTase.
Transferable analogs 5 and 6 allow the formation of appropriately
posttranslationally modified photoreactive peptide probes of isoprene
function.

REFERENCE COUNT: 82 THERE ARE 82 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 4 OF 11 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2001:848880 HCAPLUS

DOCUMENT NUMBER: 136:144964

TITLE: Statins as antioxidant therapy for preventing cardiac
myocyte hypertrophy

AUTHOR(S): Takemoto, Masao; Node, Koichi; Nakagami, Hironori;
Liao, Yulin; Grimm, Michael; Takemoto, Yaeko;
Kitakaze, Masafumi; Liao, James K.

CORPORATE SOURCE: Vascular Medicine Unit, Cardiovascular Division,
Brigham and Women's Hospital and Harvard Medical
School, Boston, MA, 02115, USA

SOURCE: Journal of Clinical Investigation (2001), 108(10),
1429-1437
CODEN: JCINAO; ISSN: 0021-9738

PUBLISHER: American Society for Clinical Investigation

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Cardiac hypertrophy is a major cause of morbidity and mortality worldwide.
The hypertrophic process is mediated, in part, by small G proteins of the
Rho family. We hypothesized that statins, inhibitors of
3-hydroxy-3-methylglutaryl-CoA reductase, inhibit cardiac hypertrophy by

blocking Rho **isoprenylation**. We treated neonatal rat cardiac myocytes with angiotensin II (AngII) with and without simvastatin (Sim) and found that Sim decreased AngII-induced protein content, [3H] leucine uptake, and atrial natriuretic factor (ANF) promoter activity. These effects were associated with decreases in cell size, **membrane** Rho activity, superoxide anion ($O_2^{\cdot-}$) production, and intracellular oxidation, and were reversed with L-mevalonate or geranylgeranylpyrophosphate, but not with farnesylpyrophosphate or cholesterol. Treatments with the Rho inhibitor C3 exotoxin and with **cell-permeable** superoxide dismutase also decreased AngII-induced $O_2^{\cdot-}$ production and myocyte hypertrophy. Overexpression of the dominant-neg. Rho mutant N17Rac1 completely inhibited AngII-induced intracellular oxidation and ANF promoter activity, while N19RhoA partially inhibited it, and N17Cdc42 had no effect. Indeed, Sim inhibited cardiac hypertrophy and decreased myocardial Rac1 activity and $O_2^{\cdot-}$ production in rats treated with AngII infusion or subjected to transaortic constriction. These findings suggest that statins prevent the development of cardiac hypertrophy through an antioxidant mechanism involving inhibition of Rac1.

REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 5 OF 11 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2001:334220 HCAPLUS

DOCUMENT NUMBER: 136:161145

TITLE: Visualization of bisphosphonate-induced caspase-3 activity in apoptotic osteoclasts in vitro

AUTHOR(S): Benford, H. L.; McGowan, N. W. A.; Helfrich, M. H.; Nuttall, M. E.; Rogers, M. J.

CORPORATE SOURCE: Department of Medicine and Therapeutics, University of Aberdeen Medical School, Aberdeen, AB25 2ZD, UK

SOURCE: Bone (New York, NY, United States) (2001), 28(5), 465-473

CODEN: BONEDL; ISSN: 8756-3282

PUBLISHER: Elsevier Science Inc.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Bisphosphonates inhibit osteoclast-mediated bone resorption by mechanisms that have only recently become clear. Whereas nitrogen-containing bisphosphonates affect osteoclast function by preventing protein prenylation (especially geranylgeranylation), non-nitrogen-containing bisphosphonates have a different mol. mechanism of action. In this study, we demonstrate that nitrogen-containing bisphosphonates (risedronate, alendronate, pamidronate, and zoledronic acid) and non-nitrogen-containing bisphosphonates (clodronate and etidronate) cause apoptosis of rabbit osteoclasts, human osteoclastoma-derived osteoclasts, and human osteoclast-like cells generated in cultures of bone marrow in vitro. Osteoclast apoptosis was shown to involve characteristic morphol. changes, loss of mitochondrial **membrane** potential, and the activation of caspase-3-like proteases capable of cleaving peptide **substrates** with the sequence DEVD. Caspase-3-like activity could be visualized in unfixed, dying osteoclasts and osteoclast-like cells using a **cell-permeable**, fluorogenic **substrate**.

Bisphosphonate-induced osteoclast apoptosis was dependent on caspase activation, because apoptosis resulting from alendronate, clodronate, or zoledronic acid treatment was suppressed by zVAD-fmk, a broad-range caspase inhibitor, or by SB-281277, a specific isatin sulfonamide inhibitor of caspase-3/-7. Furthermore, caspase-3 (but not caspase-6 or caspase-7) activity could be detected and quantitated in lysates from purified rabbit osteoclasts, whereas the p17 fragment of active caspase-3 could be detected in human osteoclast-like cells by immunofluorescence

staining. Caspase-3, therefore, appears to be the major effector caspase activated in osteoclasts by bisphosphonate treatment. Caspase activation and apoptosis induced by nitrogen-containing bisphosphonates are likely to be the consequence of the loss of geranylgeranylated rather than **farnesylated** proteins, because the ability to cause apoptosis and caspase activation was mimicked by GGTI-298, a specific inhibitor of protein geranylgeranylation, whereas FTI-277, a specific inhibitor of protein **farnesylation**, had no effect on apoptosis or caspase activity.

REFERENCE COUNT: 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 6 OF 11 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1996:584596 HCAPLUS

DOCUMENT NUMBER: 125:245313

TITLE: Prenylation of an interferon- γ -induced GTP-binding protein: the human guanylate binding protein, huGBP1

AUTHOR(S): Nantais, David E.; Schwemmle, Martin; Stickney, John T.; Vestal, Deborah J.; Buss, Janice E.

CORPORATE SOURCE: Dep. Biochemistry, Iowa State Univ., Ames, IA, 50011, USA

SOURCE: Journal of Leukocyte Biology (1996), 60(3), 423-431
CODEN: JLBIE7; ISSN: 0741-5400

PUBLISHER: Federation of American Societies for Experimental Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Interferons (IFN) and lipopolysaccharide (LPS) cause multiple changes in isoprenoid-modified proteins in murine macrophages, the most dramatic being the expression of a prenyl protein of 65 kDa. The guanylate binding proteins (GBPs) are IFN-inducible GTP-binding proteins of .apprx.65 kDa that possess a CaaX motif at their C-terminus, indicating that they might be **substrates** for prenyltransferases. The human GBP1 protein, when expressed in transfected COS-1 **cells, incorporates** radioactivity from the isoprenoid precursor [3H]mevalonate. In addition, huGBPs expressed from the endogenous genes in IFN- γ -treated human fibroblasts or monocytic cells were also isoprenoid modified. IFN- γ -induced huGBPs in HL-60 cells were not labeled by the specific C20 isoprenoid, [3H]geranylgeraniol, but did show decreased isoprenoid incorporation in cells treated with the farnesyl transferase inhibitor BZA-5B, indicating that huGBPs in HL-60 cells are probably modified by a C15 farnesyl rather than the more common C20 lipid. Differentiated HL-60 cells treated with IFN- γ /LPS showed no change in the profile of constitutive **isoprenylated** proteins and the IFN- γ /LPS-induced huGBPs remained prenylated. Despite being prenylated, huGBP1 in COS cells and endogenous huGBPs in HL-60 cells were primarily (.apprx.85%) cytosolic. Human GBPs are thus among the select group of prenyl proteins whose synthesis is tightly regulated by a cytokine. HuGBP1 is an abundant protein whose prenylation may be vulnerable to farnesyl transferase. inhibitors that are designed to prevent **farnesylation** of Ras proteins.

L6 ANSWER 7 OF 11 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1995:562867 HCAPLUS

DOCUMENT NUMBER: 123:160098

TITLE: **Bisubstrate** inhibitors of farnesyltransferase: a novel class of specific inhibitors of ras transformed cells

AUTHOR(S): Manne, Veeraswamy; Yan, Ning; Carboni, Joan M.;

Tuomai, Anne V.; Ricca, Carolyn S.; Brown, Johnni
Gullo; Andahazy, Mary L.; Schmidt, Robert J.; Patel,
Dinesh; et al.
CORPORATE SOURCE: Oncology Drug Discovery, Div. Discovery Chemistry,
Dep. Mol. Biol., Bristol-Myers Squibb Pharmaceutical
Research Institute, Princeton, NJ, 08543-4000, USA
SOURCE: Oncogene (1995), 10(9), 1763-79
CODEN: ONCNES; ISSN: 0950-9232
PUBLISHER: Stockton
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We describe the biol. properties of a new class of potent
farnesyltransferase (FT) inhibitors designed as **bisubstrate**
analog inhibitors. These inhibitors incorporate the structural motifs of
both farnesyl pyrophosphate and the CAAX tetrapeptide, the two
substrates of the reaction catalyzed by FT. Both the **phosphinate**
inhibitor, BMS-185878, and the **phosphonate** inhibitor, BMS-184467,
exhibited higher in vitro FT selectivity than some of the previously
reported CVFM peptidomimetics and benzodiazepine analogs. Xenopus oocyte
maturation induced by microinjected oncogenic Ras proteins was blocked by
coinjected BMS-184467 and BMS-185878. However, both inhibitors showed
poor cell activity presumably because of the doubly charged nature of the
comps. Thus, masking the charge on the carboxylate ion markedly improved
the **cell permeability** of BMS-185878, leading to
BMS-186511, the Me carboxyl ester prodrug. BMS-186511 inhibited FT
activity in whole cells as determined by inhibition of p21 Ras protein
processing, inhibition of **farnesylation** of proteins including
Ras and the accumulation of **unfarnesylated** Ras proteins in the
cytosolic fraction. While the cellular effects of these
bisubstrate analog inhibitors had no significant effect on growth
of untransformed NIH3T3 cells, they produced pronounced inhibition of Ras
transformed cell growth. Both the anchorage dependent and independent
growth of ras transformed cells were severely curtailed by micromolar
concns. of BMS-186511. We also found that both H-ras and K-ras
transformed cells are affected by this **bisubstrate** inhibitor.
However, K-ras transformed cells appear to be less sensitive. The
inhibition of FT activity in cells and the ensuing inhibition of ras
transformed cell growth is further manifested in distinct morphol. changes
in cells. Cells flattened, became less refractile and grew in contact
inhibited monolayer. Moreover, the highly diffused character of the actin
cytoskeleton in the ras transformed cells was dramatically reverted to an
organized network of stress cables crisscrossing the entire cells upon
treatment with BMS-186511. All of these effects of BMS-186511 are limited
to ras transformed cells that utilize **farnesylated** Ras, but are
not seen in transformed cells that use geranylgeranyl Ras or myristoyl
Ras. Significantly, these FT inhibitors did not produce any signs of
gross cytotoxicity in untransformed, ras transformed cells or other
oncogene transformed cells.

L6 ANSWER 8 OF 11 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1993:557037 HCAPLUS

DOCUMENT NUMBER: 119:157037

TITLE: Rab GDP dissociation inhibitor as a general regulator
for the **membrane** association of Rab proteins

AUTHOR(S): Ullrich, Oliver; Stenmark, Harald; Alexandrov, Kirill;
Huber, Lukas A.; Kaibuchi, Kozo; Sasaki, Takuya;
Takai, Yoshimi; Zerial, Marino

CORPORATE SOURCE: Eur. Mol. Biol. Lab., Heidelberg, D-6900, Germany

SOURCE: Journal of Biological Chemistry (1993), 268(24),
18143-50

CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Rab proteins comprise a family of small GTPases that serve a regulatory role in **membrane** traffic. These proteins are in part cytosolic and in part associated with the **membranes** of specific exocytic and endocytic organelles. Smg p25A/rab3A GDI, a cytosolic protein which inhibits the dissociation of GDP from smg p25A/rab3A, Sec4p, and rab11, has also been found to prevent association of rab3A with the **membrane**. In this study, the authors have used Madin-Darby canine kidney **cells permeabilized** with the bacterial toxin streptolysin O to test the general activity of rab3A GDI in modulating the **membrane** association of various small GTP-binding proteins. Rab3A GDP dissociation inhibitor (GDI) removed from the **membrane** all rab proteins the authors have tested and inhibited the **membrane** binding of in vitro translated rab proteins. However, rab3A GDI had a limited effect on the **membrane** association of a mutant rab5 protein which contained a **farnesylated** cysteine motif. Finally, the authors found that, although rab3A GDI resides primarily in the cytosol, it is also associated with compartments of the exocytic and endocytic pathways. Since rab3A GDI can modulate the **membrane** association of various rab proteins, the authors propose to rename it rab GDI.

L6 ANSWER 9 OF 11 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1993:140633 HCAPLUS

DOCUMENT NUMBER: 118:140633

TITLE: Nerve growth factor induces a succession of increases in **isoprenylated** methylated small

GTP-binding proteins of PC-12 pheochromocytoma cells

AUTHOR(S): Haklai, R.; Lerner, S.; Kloog, Y.

CORPORATE SOURCE: George S. Wise Fac. Life Sci., Tel Aviv Univ., Tel Aviv, 69978, Israel

SOURCE: Neuropeptides (Edinburgh, United Kingdom) (1993), 24(1), 11-25

CODEN: NRPPDD; ISSN: 0143-4179

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Pheochromocytoma (PC-12) cells exposed to nerve growth factor (NGF) acquire a sympathetic neuron-like phenotype. This NGF-response is blocked by methylation inhibitors and can be mimicked by the **farnesylated** methylated small GTP-binding protein p21ras. The implicated involvement of prenylation, methylation and a small GTP-binding protein in the NGF-response has been studied by directly measuring 3H-mevalonic acid (MVA)-**metabolites incorporated** into proteins, protein carboxyl [methyl-3H]ester formation and levels of [α -32P]GTP-binding proteins in NGF-induced PC-12 cells. NGF induces a 2-3-fold increase in 21-24 kDa methylated **membrane** proteins that incorporate 3H-MVA-metabolites, and bind GTP. Levels of [α -32P]GTP-binding in these proteins were increased by 2-3-fold. Methylation and **membrane** association of the small GTP-binding proteins were blocked by lovastatin, an inhibitor of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, which also enhanced their labeling by 3H-MVA-metabolites. Cycloheximide reduced the levels of [methyl-3H] labeled 21-24 kDa proteins and of the overlapping [α -32P]GTP binding-proteins. About 70% of the [methyl-3H]-groups found in these proteins were recovered from two dimensional gel blots in nine distinct spots of [α -32P]GTP-binding proteins. Thus, in PC-12 cells, NGF induces an increase in the synthesis of prenylated methylated small GTP-binding proteins. The efficacy of lovastatin blockage of protein methylation and enhancement of 3H-MVA-**metabolites incorporation** into GTP-binding proteins was

lower in NGF-induced cells than in controls. This suggest that NGF also induces an increase in HMG-CoA reductase activity. At the early phase of the NGF response in PC-12 cells (15 min-1 h), the levels of two small GTP-binding proteins (mol. mass of 21-22 kDa and 23-24 kDa) were increased. Thus, at least two proteins, of which one but not the other may be p21ras, appear to be involved in the early response. After a lag period of 24 h with NGF, a second more robust phase of increase in methylated small GTP-binding proteins was apparent. This relatively late response, which was almost completed within 24 h, may reflect involvement of small GTP-binding proteins in neurite-outgrowth and in the functional activity of the differentiated cells. Many small GTP-binding proteins were increased during the second phase, precluding electrophoretic separation of all of them. Three proteins, however, were well separated (one 23-24 kDa protein and two 21-22 kDa proteins). Evidently, NGF induces a succession of changes in prenylated methylated small GTP binding-proteins which are synchronized with the processes by which PC-12 cells acquire a neuron-like phenotype. Prenylated methylated and GTP-binding proteins are therefore required in these processes.

L6 ANSWER 10 OF 11 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1992:630782 HCAPLUS

DOCUMENT NUMBER: 117:230782

TITLE: **Isoprenylation** and carboxymethylation in small GTP-binding proteins of pheochromocytoma (PC-12) cells

AUTHOR(S): Lerner, S.; Haklai, R.; Kloog, Y.

CORPORATE SOURCE: Tel Aviv Univ., Tel Aviv, 69978, Israel

SOURCE: Cellular and Molecular Neurobiology (1992), 12(4), 333-51

CODEN: CMNEDI; ISSN: 0272-4340

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A group of 21-24-kDa proteins of pheochromocytoma (PC-12) cells was found in blot overlay assays to bind specifically [α -³²P]GTP. Binding was inhibited by GTP analogs but not by ATP. Such small GTP-binding proteins were found in the cytosolic and in the **particulate** fraction of the cells, but they were unevenly distributed: .apprx.75% of the small GTP-binding proteins were localized within the **particulate** fraction of the cells. Separation of these proteins by 2-dimensional gel electrophoresis revealed the existence of 7 distinct [α -³²P]GTP-binding proteins. Targeting of the small GTP-binding proteins to the **particulate** fraction of PC-12 cells requires modification by isoprenoids, since depleting the cells of the isoprenoid precursor mevalonic acid (MVA) by the use of lovastatin resulted in a 50% decrease in **membrane**-bound small GTP-binding proteins, with a proportionate increase in the cytosolic form. This blocking effect of lovastatin was reversed by exogenously added MVA. In addition, metabolic labeling of PC-12 cells with [³H]MVA revealed incorporation of [³H]MVA metabolites into the cluster of 21-24-kDa proteins in a form typical of isoprenoids; the label was not removed from the proteins by hydroxylamine, and labeling was enhanced in cells incubated with lovastatin. The latter effect reflects a decrease in the isotopic dilution of the exogenously added [³H]MVA, as the addition of exogenous MVA reversed the effect of lovastatin on [³H]MVA-**metabolite incorporation** into the 21-24-kDa proteins. Addnl. expts. demonstrated that **isoprenylation** is required not only for **membrane** association of small GTP-binding proteins, but also for their further modification by a methylation enzyme. This was evident in expts. in which the cells were metabolically labeled with [methyl-³H]methionine, a methylation precursor. The group of 21-24-kDa proteins was labeled with a methyl-³H group in a form typical of

C-terminal-cysteiny l carboxylmethyl esters. Their methylation was blocked by the methylation inhibitors methylthioadenosine (MTA), 3-deazadenosine and homocysteine thiolactone as well as by lovastatin. MVA reversed the lovastatin block of methylation. Two-dimensional gel anal. of the [3H]methylated proteins detected 7 methylated small GTP-binding proteins that correspond to the **isoprenylated** proteins. Levels of the small GTP-binding proteins as well as **isoprenylation** and methylation were reduced by cycloheximide. Distribution of the methylated proteins between **particulate** and cytosolic fractions was found to be similar to that of the small GTP-binding proteins (i.e., a 4:1 ratio). **Membrane** association of these proteins does not require methylation, as it was not blocked by MTA. **Isoprenylated** 21-24-kDa proteins were found to be equally distributed between **particulate** and cytosol, even though most of the methylated small GTP-binding proteins are **membrane** bound and their **membrane** binding depends on isoprenoid modification. The data are consistent with a scheme of events in which small GTP-binding proteins of PC-12 cells are **isoprenylated** shortly after translocation and this modification is a prerequisite for their **membrane** association and methylation. The excess of **isoprenylated** 21-24-kDa proteins in the cytosol may reflect proteins that were localized in the **membranes** and underwent demethylation and dissociation from the **membranes**. Apparently, the known requirements of isoprenoids for normal cell cycling is related, inter alia, to a requirement for **isoprenylation** of small GTP-binding proteins.

L6 ANSWER 11 OF 11 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1988:72838 HCAPLUS

DOCUMENT NUMBER: 108:72838

TITLE: **Isoprenylated** proteins in cultured cells:
subcellular distribution and changes related to
altered morphology and growth arrest induced by
mevalonate deprivation

AUTHOR(S): Maltese, William A.; Sheridan, Kathleen M.

CORPORATE SOURCE: Coll. Physicians Surg., Columbia Univ., New York, NY,
10032, USA

SOURCE: Journal of Cellular Physiology (1987), 133(3), 471-81
CODEN: JCLLAX; ISSN: 0021-9541

DOCUMENT TYPE: Journal

LANGUAGE: English

AB In the presence of lovastatin (mevinolin), an inhibitor of endogenous mevalonate synthesis, C1300 murine neuroblastoma **cells** **incorporated** [2-14C]mevalonate into several discrete polypeptides that were separable by SDS-PAGE. The electrophoretic pattern of the labeled proteins did not vary substantially when cells were homogenized with Ca²⁺, Mg²⁺, high concns. of NaCl or phosphatase inhibitor, or when cells were lysed immediately in TCA. When cells that had been prelabeled with [14C]mevalonate were incubated with lovastatin and simultaneously deprived of exogenous mevalonate, there was a 50-60% decline in the concentration of protein-bound isoprenoid label within 17 h. In contrast, there was little change in the radioactivity in the sterol, dolichol, or ubiquinone fractions. The time course of the decline in mevalonate-derived label in cellular polypeptides paralleled the onset of neurite outgrowth and preceded the decline of DNA synthesis, suggesting that a decreased intracellular concentration of protein-bound isoprenoid groups may contribute to the well-documented effects of mevalonate deprivation on cell morphol. and cell cycling. Fractionation of neuroblastoma cells by differential centrifugation and sucrose d.-gradient centrifugation revealed that

mevalonate-labeled proteins of 53 kilodaltons (kDa), 22-26 kDa, and 17 kDa were concentrated in the cytosol. Proteins migrating at 45 kDa were found in both the soluble and **particulate** fractions, including those enriched in mitochondria and plasma **membrane**. The **isoprenylated** proteins migrating at .apprx.66 kDa were localized exclusively in the nuclear fraction. When chromatin was removed from the nuclei by extraction with 2M NaCl, the 66-kDa **isoprenylated** proteins remained associated with the residual components of the nuclear matrix and lamina. **Isoprenylated** proteins with electrophoretic mobilities similar to those observed in neuroblastoma cells were detected in a variety of established cell lines. However, there was considerable variation among cell lines in the overall efficiency of protein labeling with [14C]mevalonate and in the prominence and mobilities of specific labeled proteins in the 45-70-kDa range. Comparisons of paired transformed vs. nontransformed fibroblast cell lines suggested that the profile of mevalonate-labeled proteins in a given cell line is not altered by malignant transformation. The finding that electrophoretically distinct **isoprenylated** proteins are localized in discrete subcellular compartments indicates that they do not represent dissociated subunits of a single multimeric proteins complex, and provides a possible explanation for the diverse effects of mevalonate deprivation. The presence of these proteins in a wide variety of mammalian cells suggests that they play a fundamental role in cell structure and(or) regulation.

=> d que stat 18

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L1      2178 SEA FILE=HCAPLUS ABB=ON  ?FARNESYLAT? OR ?ISOPRENYLAT?
L2      2 SEA FILE=HCAPLUS ABB=ON  L1 AND (?AZID?(3A)?FARNESYL? OR
      TAG?(2W)?AZID?(W)?SUBSTRAT? OR TAS OR ?AZID?(3A)TAG?)
L3      919 SEA FILE=HCAPLUS ABB=ON  L1 AND ((?PHOSPHIN?(3A)?CAPTUR?(W)(?RE
      AGENT? OR ?REACT?)) OR ?SUBSTRAT? OR ?SOLID?(W)(?PHASE? OR
      ?SUPPORT?) OR ?BEAD? OR ?RESIN? OR ?MEMBRAN? OR ?PARTICL? OR
      ?PARTICUL? OR ?TRIPHENYLPHOSPHIN? OR ?IMINOPHOSPHORAN? OR
      ?STAUDINGER?)
L4      15 SEA FILE=HCAPLUS ABB=ON  L1 AND (?METABOL?(W)?INCORPOR? OR
      ?CELL?(W)(?UPTAK? OR ?PERMEAB? OR ?INCORPORAT?) OR IN(W)?VIVO?(
      W)?LABEL?)
L5      10 SEA FILE=HCAPLUS ABB=ON  L3 AND L4
L6      11 SEA FILE=HCAPLUS ABB=ON  L2 OR L5
L7      36 SEA L6
L8      15 DUP REMOV L7 (21 DUPLICATES REMOVED)

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=> d ibib abs 18 1-15

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L8      ANSWER 1 OF 15      MEDLINE on STN      DUPLICATE 1
ACCESSION NUMBER: 2004422833      MEDLINE
DOCUMENT NUMBER:  PubMed ID: 15308774
TITLE:          A tagging-via-substrate technology for detection
                and proteomics of farnesylated proteins.
AUTHOR:         Kho Yoonjung; Kim Sung Chan; Jiang Chen; Barma Deb; Kwon
                Sung Won; Cheng Jinke; Jaunbergs Janis; Weinbaum Carolyn;
                Tamanoi Fuyuhiko; Falck John; Zhao Yingming
CORPORATE SOURCE: Department of Biochemistry, University of Texas
                Southwestern Medical Center, Dallas, TX 75390-9038.
CONTRACT NUMBER: CA 41996 (NCI)
                CA 85146 (NCI)
                CA32737 (NCI)
                GM 31278 (NIGMS)
                GM 56372 (NIGMS)
SOURCE:         Proceedings of the National Academy of Sciences of the
                United States of America, (2004 Aug 24) 101 (34) 12479-84.
                Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY:   United States
DOCUMENT TYPE:   Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:       English
FILE SEGMENT:   Priority Journals
ENTRY MONTH:    200410
ENTRY DATE:     Entered STN: 20040826
                Last Updated on STN: 20041019
                Entered Medline: 20041018
AB      A recently developed proteomics strategy, designated tagging-via-
substrate (TAS) approach, is described for the detection
and proteomic analysis of farnesylated proteins.  TAS
technology involves metabolic incorporation of a
synthetic azido-farnesyl analog and chemoselective
derivatization of azido-farnesyl-modified proteins by
an elegant version of Staudinger reaction, pioneered by the
Bertozzi group, using a biotinylated phosphine capture
reagent. The resulting protein conjugates can be specifically
detected and/or affinity-purified by streptavidin-linked horseradish
peroxidase or agarose beads, respectively. Thus, the technology
enables global profiling of farnesylated proteins by enriching
farnesylated proteins and reducing the complexity of
farnesylation subproteome. Azido-farnesylated
proteins maintain the properties of protein farnesylation,

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including promoting **membrane** association, Ras-dependent mitogen-activated protein kinase kinase activation, and inhibition of lovastatin-induced apoptosis. A proteomic analysis of **farnesylated** proteins by TAS technology revealed 18 **farnesylated** proteins, including those with potentially novel **farnesylation** motifs, suggesting that future use of this method is likely to yield novel insight into protein **farnesylation**. TAS technology can be extended to other posttranslational modifications, such as geranylgeranylation and myristoylation, thus providing powerful tools for detection, quantification, and proteomic analysis of posttranslationally modified proteins.

L8 ANSWER 2 OF 15 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2004273246 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15173010
TITLE: Apoptotic and cytostatic farnesyltransferase inhibitors have distinct pharmacology and efficacy profiles in tumor models.
COMMENT: Erratum in: Cancer Res. 2004 Oct 15;64(20):7645
AUTHOR: Manne Veeraswamy; Lee Francis Y F; Bol David K; Gullo-Brown Johnni; Fairchild Craig R; Lombardo Louis J; Smykla Richard A; Vite Gregory D; Wen Mei-Li D; Yu Chiang; Wong Tai Wai; Hunt John T
CORPORATE SOURCE: Oncology Drug Discovery and Discovery Chemistry, Bristol-Myers Squibb Company Pharmaceutical Research Institute, Princeton, New Jersey 08543, USA.. Veeraswamy.manne@bms.com
SOURCE: Cancer research, (2004 Jun 1) 64 (11) 3974-80. Journal code: 2984705R. ISSN: 0008-5472.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200407
ENTRY DATE: Entered STN: 20040603
Last Updated on STN: 20040728
Entered Medline: 20040726

AB BMS-214662 and BMS-225975 are tetrahydrobenzodiazepine-based farnesyltransferase inhibitors (FTIs) that have nearly identical structures and very similar pharmacological profiles associated with farnesyltransferase (FT) inhibition. Despite their similar activity against FT in vitro and in cells, these compounds differ dramatically in their apoptotic potency and tumor-regressing activity in vivo. BMS-214662 is the most potent apoptotic FTI known and exhibits curative responses in mice bearing a variety of staged human tumor xenografts such as HCT-116 human colon tumor. By contrast, BMS-225975 does not cause tumor regression and at best causes partial tumor growth inhibition in staged HCT-116 human colon tumor xenografts. Lack of tumor regression activity in BMS-225975 was attributable to its relatively weak apoptotic potency, not to poor **cell permeability** or pharmacokinetics. Both compounds were equally effective in inhibiting Ras processing and causing accumulation of a variety of **nonfarnesylated substrates** of FT in HCT-116 cells. Because BMS-225975 has poor apoptotic activity compared with BMS-214662 but inhibits FT to the same extent as BMS-214662, it is very unlikely that FT inhibition alone can account for the apoptotic potency of BMS-214662. Clearly distinct patterns of sensitivities in a cell line panel were obtained for the apoptotic FTI BMS-214662 and the cytostatic FTI BMS-225975. Activation of the c-Jun-NH(2)-terminal kinase pathway was readily observed with BMS-214662 but not with BMS-225975. We developed a highly sensitive San-1

murine xenograft tumor model that is **particularly** useful for evaluating the in vivo activity of cytostatic FTIs such as BMS-225975.

L8 ANSWER 3 OF 15 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2001668940 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11714734
TITLE: Statins as antioxidant therapy for preventing cardiac myocyte hypertrophy.
AUTHOR: Takemoto M; Node K; Nakagami H; Liao Y; Grimm M; Takemoto Y; Kitakaze M; Liao J K
CORPORATE SOURCE: Vascular Medicine Unit, Cardiovascular Division, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115, USA.
CONTRACT NUMBER: HL-48743 (NHLBI)
HL-52233 (NHLBI)
HL-62602 (NHLBI)
SOURCE: Journal of clinical investigation, (2001 Nov) 108 (10) 1429-37.
Journal code: 7802877. ISSN: 0021-9738.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011121
Last Updated on STN: 20020123
Entered Medline: 20011213
AB Cardiac hypertrophy is a major cause of morbidity and mortality worldwide. The hypertrophic process is mediated, in part, by small G proteins of the Rho family. We hypothesized that statins, inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase, inhibit cardiac hypertrophy by blocking Rho **isoprenylation**. We treated neonatal rat cardiac myocytes with angiotensin II (AngII) with and without simvastatin (Sim) and found that Sim decreased AngII-induced protein content, [3H] leucine uptake, and atrial natriuretic factor (ANF) promoter activity. These effects were associated with decreases in cell size, **membrane** Rho activity, superoxide anion (O₂⁻) production, and intracellular oxidation, and were reversed with L-mevalonate or geranylgeranylpyrophosphate, but not with farnesylpyrophosphate or cholesterol. Treatments with the Rho inhibitor C3 exotoxin and with **cell-permeable** superoxide dismutase also decreased AngII-induced O₂⁻ production and myocyte hypertrophy. Overexpression of the dominant-negative Rho mutant N17Rac1 completely inhibited AngII-induced intracellular oxidation and ANF promoter activity, while N19RhoA partially inhibited it, and N17Cdc42 had no effect. Indeed, Sim inhibited cardiac hypertrophy and decreased myocardial Rac1 activity and O₂⁻ production in rats treated with AngII infusion or subjected to transaortic constriction. These findings suggest that statins prevent the development of cardiac hypertrophy through an antioxidant mechanism involving inhibition of Rac1.

L8 ANSWER 4 OF 15 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2001241872 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11344045
TITLE: Visualization of bisphosphonate-induced caspase-3 activity in apoptotic osteoclasts in vitro.
AUTHOR: Benford H L; McGowan N W; Helfrich M H; Nuttall M E; Rogers M J
CORPORATE SOURCE: Department of Medicine and Therapeutics, University of Aberdeen Medical School, Foresterhill, Aberdeen, AB25 2ZD,

UK.
 SOURCE: Bone, (2001 May) 28 (5) 465-73.
 Journal code: 8504048. ISSN: 8756-3282.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 200108
 ENTRY DATE: Entered STN: 20010813
 Last Updated on STN: 20010813
 Entered Medline: 20010809

AB Bisphosphonates inhibit osteoclast-mediated bone resorption by mechanisms that have only recently become clear. Whereas nitrogen-containing bisphosphonates affect osteoclast function by preventing protein prenylation (especially geranylgeranylation), non-nitrogen-containing bisphosphonates have a different molecular mechanism of action. In this study, we demonstrate that nitrogen-containing bisphosphonates (risedronate, alendronate, pamidronate, and zoledronic acid) and non-nitrogen-containing bisphosphonates (clodronate and etidronate) cause apoptosis of rabbit osteoclasts, human osteoclastoma-derived osteoclasts, and human osteoclast-like cells generated in cultures of bone marrow in vitro. Osteoclast apoptosis was shown to involve characteristic morphological changes, loss of mitochondrial **membrane** potential, and the activation of caspase-3-like proteases capable of cleaving peptide **substrates** with the sequence DEVD. Caspase-3-like activity could be visualized in unfixed, dying osteoclasts and osteoclast-like cells using a **cell-permeable**, fluorogenic **substrate**. Bisphosphonate-induced osteoclast apoptosis was dependent on caspase activation, because apoptosis resulting from alendronate, clodronate, or zoledronic acid treatment was suppressed by zVAD-fmk, a broad-range caspase inhibitor, or by SB-281277, a specific isatin sulfonamide inhibitor of caspase-3/-7. Furthermore, caspase-3 (but not caspase-6 or caspase-7) activity could be detected and quantitated in lysates from purified rabbit osteoclasts, whereas the p17 fragment of active caspase-3 could be detected in human osteoclast-like cells by immunofluorescence staining. Caspase-3, therefore, appears to be the major effector caspase activated in osteoclasts by bisphosphonate treatment. Caspase activation and apoptosis induced by nitrogen-containing bisphosphonates are likely to be the consequence of the loss of geranylgeranylated rather than **farnesylated** proteins, because the ability to cause apoptosis and caspase activation was mimicked by GGTI-298, a specific inhibitor of protein geranylgeranylation, whereas FTI-277, a specific inhibitor of protein **farnesylation**, had no effect on apoptosis or caspase activity.

L8 ANSWER 5 OF 15 MEDLINE on STN
 ACCESSION NUMBER: 2000265338 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 10807058
 TITLE: Mitogenic effect of lithium in FRTL-5 cells can be reversed by blocking de novo cholesterol synthesis and subsequent signal transduction.
 AUTHOR: Tasevski V; Benn D; King M; Luttrell B; Simpson A
 CORPORATE SOURCE: Department of Endocrinology, Royal North Shore Hospital, St. Leonards, NSW, Australia..
 vtasevsk@doh.health.nsw.gov.au
 SOURCE: Thyroid : official journal of the American Thyroid Association, (2000 Apr) 10 (4) 305-11.
 Journal code: 9104317. ISSN: 1050-7256.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000810
Last Updated on STN: 20000810
Entered Medline: 20000725

AB Lithium therapy is the therapeutic mainstay for bipolar disorder and has been associated in the thyroid with euthymic goiter, hyper and hypothyroidism as well as thyroid autoimmune disease. The FRTL-5 cell line is a well known model of thyroid cell physiology, where lithium has been shown to increase 3H-thymidine uptake at concentrations of 2 mM. This mitogenic effect was not associated with adenylate cyclase as measured by cyclic adenosine monophosphate (cAMP) production. The de novo synthesis of cholesterol is an important signal transduction pathway in FRTL-5 cells, where newly synthesized Rho GTPase is geranylgeranylated, enabling **membrane** localization of the G-protein and subsequent G1 to S-phase transition, resulting from extracellular stimulation. Here we confirm lithium mitogenicity at therapeutically relevant concentrations (1 mM) and demonstrate a lithium-associated accumulation of FRTL-5 cells in S-phase of the cell cycle. These effects could be abolished by Pravastatin, a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA), the rate-limiting enzyme in the formation of intermediates (de novo cholesterol synthesis) required for G-protein prenylation. Pravastatin, similar to lithium, showed no effect on cAMP production either under basal or thyroid stimulating hormone (TSH)-stimulated conditions indicating that de novo cholesterol synthesis is not involved with adenylate cyclase. The inhibitory effect of pravastatin could be overcome by reinitiating de novo cholesterol synthesis. This was achieved by the addition of the **cell permeable**, first metabolite (mevalonate) after HMG-CoA, which allowed the cycle to continue, leading eventually to protein prenylation, despite the presence of Pravastatin. These novel findings demonstrate lithium involvement in de novo cholesterol synthesis and G-protein prenylation, an important signal transduction pathway in FRTL-5 cells.

L8 ANSWER 6 OF 15 MEDLINE on STN
ACCESSION NUMBER: 1999349894 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10423031
TITLE: Bisphosphonates: from the laboratory to the clinic and back again.
AUTHOR: Russell R G; Rogers M J
CORPORATE SOURCE: Division of Biochemical and Musculoskeletal Metabolism, Human Metabolism and Clinical Biochemistry, University of Sheffield Medical School, UK.
SOURCE: Bone, (1999 Jul) 25 (1) 97-106. Ref: 106
Journal code: 8504048. ISSN: 8756-3282.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19991005
Last Updated on STN: 19991005
Entered Medline: 19990923

AB Bisphosphonates (BPs) used as inhibitors of bone resorption all contain two phosphonate groups attached to a single carbon atom, forming a "P-C-P" structure. The bisphosphonates are therefore stable analogues of naturally occurring pyrophosphate-containing compounds, which now helps to

explain their intracellular as well as their extracellular modes of action. Bisphosphonates adsorb to bone mineral and inhibit bone resorption. The mode of action of bisphosphonates was originally ascribed to physico-chemical effects on hydroxyapatite crystals, but it has gradually become clear that cellular effects must also be involved. The marked structure-activity relationships observed among more complex compounds indicate that the pharmacophore required for maximal activity not only depends upon the bisphosphonate moiety but also on key features, e.g., nitrogen substitution in alkyl or heterocyclic side chains. Several bisphosphonates (e.g., etidronate, clodronate, pamidronate, alendronate, tiludronate, risedronate, and ibandronate) are established as effective treatments in clinical disorders such as Paget's disease of bone, myeloma, and bone metastases. Bisphosphonates are also now well established as successful antiresorptive agents for the prevention and treatment of osteoporosis. In **particular**, etidronate and alendronate are approved as therapies in many countries, and both can increase bone mass and produce a reduction in fracture rates to approximately half of control rates at the spine, hip, and other sites in postmenopausal women. In addition to inhibition of osteoclasts, the ability of bisphosphonates to reduce the activation frequency and birth rates of new bone remodeling units, and possibly to enhance osteon mineralisation, may also contribute to the reduction in fractures. The clinical pharmacology of bisphosphonates is characterized by low intestinal absorption, but highly selective localization and retention in bone. Significant side effects are minimal. Current issues with bisphosphonates include the introduction of new compounds, the choice of therapeutic regimen (e.g., the use of intermittent dosing rather than continuous), intravenous vs. oral therapy, the optimal duration of therapy, the combination with other drugs, and extension of their use to other conditions, including steroid-associated osteoporosis, male osteoporosis, arthritis, and osteopenic disorders in childhood. Bisphosphonates inhibit bone resorption by being selectively taken up and adsorbed to mineral surfaces in bone, where they interfere with the action of osteoclasts. It is likely that bisphosphonates are internalized by osteoclasts and interfere with specific biochemical processes and induce apoptosis. The molecular mechanisms by which these effects are brought about are becoming clearer. Recent studies show that bisphosphonates can be classified into at least two groups with different modes of action. Bisphosphonates that closely resemble pyrophosphate (such as clodronate and etidronate) can be **metabolically incorporated** into nonhydrolysable analogues of ATP that may inhibit ATP-dependent intracellular enzymes. The more potent, nitrogen-containing bisphosphonates (such as pamidronate, alendronate, risedronate, and ibandronate) are not metabolized in this way but can inhibit enzymes of the mevalonate pathway, thereby preventing the biosynthesis of isoprenoid compounds that are essential for the posttranslational modification of small GTPases. The inhibition of protein prenylation and the disruption of the function of these key regulatory proteins explains the loss of osteoclast activity and induction of apoptosis. These different modes of action might account for subtle differences between compounds in terms of their clinical effects. In conclusion, bisphosphonates are now established as an important class of drugs for the treatment of bone diseases, and their mode of action is being unravelled. As a result, their full therapeutic potential is gradual

L8 ANSWER 7 OF 15 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 96427476 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8830800
TITLE: Prenylation of an interferon-gamma-induced GTP-binding protein: the human guanylate binding protein, huGBP1.

AUTHOR: Nantais D E; Schwemmler M; Stickney J T; Vestal D J; Buss J E
CORPORATE SOURCE: Department of Biochemistry and Biophysics, Iowa State University, Ames 50011, USA.
CONTRACT NUMBER: CA51890 (NCI)
SOURCE: Journal of leukocyte biology, (1996 Sep) 60 (3) 423-31.
Journal code: 8405628. ISSN: 0741-5400.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 20000303
Entered Medline: 19961105

AB Interferons (IFN) and lipopolysaccharide (LPS) cause multiple changes in isoprenoid-modified proteins in murine macrophages, the most dramatic being the expression of a prenyl protein of 65 kDa. The guanylate binding proteins (GBPs) are IFN-inducible GTP-binding proteins of approximately 65 kDa that possess a CaaX motif at their C-terminus, indicating that they might be **substrates** for prenyltransferases. The human GBP1 protein, when expressed in transfected COS-1 cells, **incorporates** radioactivity from the isoprenoid precursor [3H]mevalonate. In addition, huGBPs expressed from the endogenous genes in IFN-gamma-treated human fibroblasts or monocytic cells were also found to be isoprenoid modified. IFN-gamma-induced huGBPs in HL-60 cells were not labeled by the specific C20 isoprenoid, [3H]geranylgeraniol, but did show decreased isoprenoid incorporation in cells treated with the farnesyl transferase inhibitor BZA-5B, indicating that huGBPs in HL-60 cells are probably modified by a C15 farnesyl rather than the more common C20 lipid. Differentiated HL-60 cells treated with IFN-gamma/LPS showed no change in the profile of constitutive **isoprenylated** proteins and the IFN-gamma/LPS-induced huGBPs remained prenylated. Despite being prenylated, huGBP1 in COS cells and endogenous huGBPs in HL-60 cells were primarily (approximately 85%) cytosolic. Human GBPs are thus among the select group of prenyl proteins whose synthesis is tightly regulated by a cytokine. HuGBP1 is an abundant protein whose prenylation may be vulnerable to farnesyl transferase inhibitors that are designed to prevent **farnesylation** of Ras proteins.

L8 ANSWER 8 OF 15 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 95273093 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7753553
TITLE: **Bisubstrate** inhibitors of farnesyltransferase: a novel class of specific inhibitors of ras transformed cells.
AUTHOR: Manne V; Yan N; Carboni J M; Tuomari A V; Ricca C S; Brown J G; Andahazy M L; Schmidt R J; Patel D; Zahler R; +
CORPORATE SOURCE: Division of Discovery Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey 08543-4000, USA.
SOURCE: Oncogene, (1995 May 4) 10 (9) 1763-79.
Journal code: 8711562. ISSN: 0950-9232.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199506
ENTRY DATE: Entered STN: 19950629
Last Updated on STN: 20000303

Entered Medline: 19950619

AB We describe the biological properties of a new class of potent farnesyltransferase (FT) inhibitors designed as **bisubstrate** analog inhibitors. These inhibitors incorporate the structural motifs of both farnesyl pyrophosphate and the CAAX tetrapeptide, the two **substrates** of the reaction catalyzed by FT. Both the phosphinate inhibitor, BMS-185878, and the phosphonate inhibitor, BMS-184467, exhibited higher in vitro FT selectivity than some of the previously reported CVFM peptidomimetics and benzodiazepine analogs. Xenopus oocyte maturation induced by microinjected oncogenic Ras proteins was blocked by coinjected BMS-184467 and BMS-185878. However, both inhibitors showed poor cell activity presumably because of the doubly charged nature of the compounds. Thus, masking the charge on the carboxylate ion markedly improved the **cell permeability** of BMS-185878, leading to BMS-186511, the methyl carboxyl ester prodrug. BMS-186511 inhibited FT activity in whole cells as determined by inhibition of p21 Ras protein processing, inhibition of **farnesylation** of proteins including Ras and the accumulation of **unfarnesylated** Ras proteins in the cytosolic fraction. While the cellular effects of these **bisubstrate** analog inhibitors had no significant effect on growth of untransformed NIH3T3 cells, they produced pronounced inhibition of Ras transformed cell growth. Both the anchorage dependent and independent growth of ras transformed cells were severely curtailed by micromolar concentrations of BMS-186511. We also found that both H-ras and K-ras transformed cells are affected by this **bisubstrate** inhibitor. However, K-ras transformed cells appear to be less sensitive. The inhibition of FT activity in cells and the ensuing inhibition of ras transformed cell growth is further manifested in distinct morphological changes in cells. Cells flattened, became less refractile and grew in contact inhibited monolayer. Moreover, the highly diffused character of the actin cytoskeleton in the ras transformed cells was dramatically reverted to an organized network of stress cables crisscrossing the entire cells upon treatment with BMS-186511. All of these effects of BMS-186511 are limited to ras transformed cells that utilize **farnesylated** Ras, but are not seen in transformed cells that use geranylgeranyl Ras or myristoyl Ras. Significantly, these FT inhibitors did not produce any signs of gross cytotoxicity in untransformed, ras transformed cells or other oncogene transformed cells.

L8 ANSWER 9 OF 15 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
ACCESSION NUMBER: 1995:365243 BIOSIS
DOCUMENT NUMBER: PREV199598379543
TITLE: A rapid and convenient filter-binding assay for ras p21 processing enzyme farnesyltransferase.
AUTHOR(S): Khan, Sikandar G.; Mukhtar, Hasan; Agarwal, Rajesh [Reprint author]
CORPORATE SOURCE: Dep. Dermatol., Skin Diseases Res. Cent., Univ. Hosp. Cleveland, Case Western Reserve Univ., Cleveland, OH 44106, USA
SOURCE: Journal of Biochemical and Biophysical Methods, (1995) Vol. 30, No. 2-3, pp. 133-144.
CODEN: JBBMDG. ISSN: 0165-022X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Aug 1995
Last Updated on STN: 30 Aug 1995

AB Because it is the target for the development of anti-cancer agents, the mammalian cytosolic enzyme farnesyltransferase (FTase) has received significant attention in recent years. FTase catalyzes the transfer of a

farnesyl group from farnesylpyrophosphate (FPP) to cysteine-185/186 at the carboxyl terminal end of ras proteins (ras p21), a reaction essential for the localization of ras p21 to the plasma membrane for their cellular functions including cell transformation in case of oncogenic ras p21. Here, we report the development of a rapid and convenient assay procedure for FTase using phosphocellulose paper which has a binding affinity for proteins. The FTase is assayed as the transfer of (3H)farnesyl group from (3H)FPP to the ras p21 at pH 7.4 and 37 degree C in the presence of rat brain cytosol followed by the binding of radioactive **farnesylated** **Tas** p21 to the phosphocellulose paper. The radioactivity associated with ras p21 bound to the phosphocellulose paper was determined by scintillation counting after soaking the paper in trichloroacetic acid and washing with distilled water. Utilizing (3H)FPP and recombinant Ha-ras p21 as substrates in the reaction, the FTase followed Michaelis-Menten kinetics with K-m values of 1.0 and 7.69 μ -M for respectively (3H)FPP and recombinant Ha-ras p21. The method reported here has the advantages over the other published assay procedures of being rapid, convenient and economical, and can be successfully used for the basic assaying of FTase in different organs and distinct species and for the screening of novel inhibitors of FTase.

L8 ANSWER 10 OF 15 MEDLINE on STN DUPLICATE 7
 ACCESSION NUMBER: 93352635 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 8349690
 TITLE: Rab GDP dissociation inhibitor as a general regulator for the **membrane** association of rab proteins.
 AUTHOR: Ullrich O; Stenmark H; Alexandrov K; Huber L A; Kaibuchi K; Sasaki T; Takai Y; Zerial M
 CORPORATE SOURCE: European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany.
 SOURCE: Journal of biological chemistry, (1993 Aug 25) 268 (24) 18143-50.
 Journal code: 2985121R. ISSN: 0021-9258.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199309
 ENTRY DATE: Entered STN: 19931001
 Last Updated on STN: 20000303
 Entered Medline: 19930916

AB Rab proteins comprise a family of small GTPases that serve a regulatory role in **membrane** traffic. These proteins are in part cytosolic and in part associated with the **membranes** of specific exocytic and endocytic organelles. Smg p25A/rab3A GDI, a cytosolic protein which inhibits the dissociation of GDP from smg p25A/rab3A, Sec4p, and rab11, has also been found to prevent association of rab3A with the **membrane**. In this study, we have used Madin-Darby canine kidney cells permeabilized with the bacterial toxin streptolysin O to test the general activity of rab3A GDI in modulating the **membrane** association of various small GTP-binding proteins. Rab3A GDP dissociation inhibitor (GDI) removed from the **membrane** all rab proteins we have tested and inhibited the **membrane** binding of in vitro translated rab proteins. However, rab3A GDI had a limited effect on the **membrane** association of a mutant rab5 protein which contained a **farnesylated** cysteine motif. Finally, we found that, although rab3A GDI resides primarily in the cytosol, it is also associated with compartments of the exocytic and endocytic pathways. Since rab3A GDI can modulate the **membrane** association of various rab proteins, we propose to rename it rab GDI.

L8 ANSWER 11 OF 15 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 93156934 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8429920
TITLE: Nerve growth factor induces a succession of increases in **isoprenylated** methylated small GTP-binding proteins of PC-12 pheochromocytoma cells.
AUTHOR: Haklai R; Lerner S; Kloog Y
CORPORATE SOURCE: Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel.
SOURCE: Neuropeptides, (1993 Jan) 24 (1) 11-25.
Journal code: 8103156. ISSN: 0143-4179.
PUB. COUNTRY: SCOTLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199303
ENTRY DATE: Entered STN: 19930326
Last Updated on STN: 20000303
Entered Medline: 19930311

AB Pheochromocytoma (PC-12) cells exposed to nerve growth factor (NGF) acquire a sympathetic neuron-like phenotype. This NGF-response is blocked by methylation inhibitors and can be mimicked by the **farnesylated** methylated small GTP-binding protein p21ras. The implicated involvement of prenylation, methylation and a small GTP-binding protein in the NGF-response has been studied by directly measuring 3H-mevalonic acid (MVA)-**metabolites incorporated** into proteins, protein carboxy [methyl-3H]ester formation and levels of [alpha-32P]GTP-binding proteins in NGF-induced PC-12 cells. We demonstrate that NGF induces a 2-3-fold increase in 21-24 kDa methylated **membrane** proteins that incorporate 3H-MVA-metabolites, and bind GTP. Levels of [alpha-32P]GTP-binding in these proteins were increased by 2-3-fold. Methylation and **membrane** association of the small GTP-binding proteins were blocked by lovastatin, an inhibitor of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, which also enhanced their labeling by 3H-MVA-metabolites. Cycloheximide reduced the levels of [methyl-3H] labeled 21-24 kDa proteins and of the overlapping [alpha-32P]GTP binding-proteins. About 70% of the [methyl-3H]-groups found in these proteins were recovered from two dimensional gel blots in nine distinct spots of [alpha-32P]GTP-binding proteins. Taken together these results strongly suggest that in PC-12 cells, NGF induces an increase in the synthesis of prenylated methylated small GTP-binding proteins. The efficacy of lovastatin blockage of protein methylation and enhancement of 3H-MVA-**metabolites incorporation** into GTP-binding proteins was lower in NGF-induced cells than in controls. This suggests that NGF also induces an increase in HMG-CoA reductase activity. At the early phase of the NGF response in PC-12 cells (15 min-1 h), the levels of two small GTP-binding proteins (molecular mass of 21-22 kDa and 23-24 kDa) were increased. Thus, at least two proteins, of which one but not the other may be p21ras, appear to be involved in the early response. After a lag period of 24 h with NGF, a second more robust phase of increase in methylated small GTP-binding proteins was apparent. This relatively late response, which was almost completed within 24 h, may reflect involvement of small GTP-binding proteins in neurite-outgrowth and in the functional activity of the differentiated cells. Many small GTP-binding proteins were increased during the second phase, precluding electrophoretic separation of all of them. 3 proteins, however, were well separated (one 23-24 kDa protein and two 21-22 kDa proteins). (ABSTRACT TRUNCATED AT 400 WORDS)

L8 ANSWER 12 OF 15 MEDLINE on STN
ACCESSION NUMBER: 93015866 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1400319
TITLE: Post-translational modifications of p21rho proteins.
AUTHOR: Adamson P; Marshall C J; Hall A; Tilbrook P A
CORPORATE SOURCE: Section of Cell and Molecular Biology, Chester Beatty Laboratories, London, United Kingdom.
SOURCE: Journal of biological chemistry, (1992 Oct 5) 267 (28) 20033-8.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199211
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 20000303
Entered Medline: 19921116

AB Post-translational modifications of the ras proteins, which are required for plasma **membrane** localization and biological function of the proteins, have been shown to include prenylation and carboxymethylation at the carboxyl terminal cysteine residue of the cysteine-aliphatic amino acid-aliphatic amino acid-any amino acid (CAAX) box. In addition, p21Ha-ras and p21N-ras, but not p21K-ras (B), are palmitoylated. The three mammalian rho proteins (A, B, and C) are also members of the ras superfamily but have distinct biological activities and different intracellular distributions from p21ras. Analysis showed all three rho proteins are modified by a COOH-terminal carboxymethylation similar to p21ras, whereas p21rhoC labeled with [3H]mevalonic acid in vivo revealed the presence of a C20 prenyl, similar to that already described for p21rhoA. However, in vivo and in vitro studies of p21rhoB showed this protein to be modified by both C15 and C20 prenyls. Mutation of C193 in the CAAX box abolished prenylation, whereas mutation of the adjacent C192 resulted in a significant reduction in the amount of the C20, but not C15 prenyl, recovered from p21rhoB. **In vivo labeling** studies with [3H]palmitic acid and mutational analysis showed that both cysteine residues at 189 and 192 upstream of the CAAX box in p21rhoB are sites for palmitoylation. We conclude that there are different populations of post-translationally modified p21rhoB in the cell and that the sequence specificity for geranylgeranyl- and farnesyltransferases may be more complicated than previously proposed.

L8 ANSWER 13 OF 15 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 93008172 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1394371
TITLE: **Isoprenylation** and carboxymethylation in small GTP-binding proteins of pheochromocytoma (PC-12) cells.
AUTHOR: Lerner S; Haklai R; Kloog Y
CORPORATE SOURCE: Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel.
SOURCE: Cellular and molecular neurobiology, (1992 Aug) 12 (4) 333-51.
Journal code: 8200709. ISSN: 0272-4340.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199211
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 20000303

Entered Medline: 19921113

AB 1. A group of 21 to 24-kDa proteins of pheochromocytoma (PC-12) cells was found in blot overlay assays to bind specifically [alpha-32P]GTP. Binding was inhibited by GTP analogues but not by ATP. Such small GTP-binding proteins were found in the cytosolic and in the **particulate** fraction of the cells, but they were unevenly distributed: about 75% of the small GTP-binding proteins were localized within the **particulate** fraction of the cells. Separation of these proteins by two-dimensional gel electrophoresis revealed the existence of seven distinct [alpha-32P]GTP-binding proteins. 2. Targeting of the small GTP-binding proteins to the **particulate** fraction of PC-12 cells requires modification by isoprenoids, since depleting the cells of the isoprenoid precursor mevalonic acid (MVA) by the use of lovastatin resulted in a 50% decrease in **membrane**-bound small GTP-binding proteins, with a proportionate increase in the cytosolic form. This blocking effect of lovastatin was reversed by exogenously added MVA. 3. In addition, metabolic labeling of PC-12 cells with [3H]MVA revealed incorporation of [3H]MVA metabolites into the cluster of 21 to 24-kDa proteins in a form typical of isoprenoids; the label was not removed from the proteins by hydroxylamine, and labeling was enhanced in cells incubated with lovastatin. The latter effect reflects a decrease in the isotopic dilution of the exogenously added [3H]MVA, as the addition of exogenous MVA reversed the effect of lovastatin on [3H]MVA-**metabolite incorporation** into the 21 to 24-kDa proteins. 4. Additional experiments demonstrated that **isoprenylation** is required not only for **membrane** association of small GTP-binding proteins, but also for their further modification by a methylation enzyme. This was evident in experiments in which the cells were metabolically labeled with [methyl-3H]methionine, a methylation precursor. The group of 21 to 24-kDa proteins was labeled with a methyl-3H group in a form typical of C-terminal-cysteiny carboxylmethyl esters. Their methylation was blocked by the methylation inhibitors methylthioadenosine (MTA), 3-deazadenosine and homocysteine thiolactone as well as by lovastatin. MVA reversed the lovastatin block of methylation. 5. Two-dimensional gel analysis of the [3H]methylated proteins detected seven methylated small GTP-binding proteins that correspond to the **isoprenylated** proteins. Levels of the small GTP-binding proteins as well as **isoprenylation** and methylation were reduced by cycloheximide. 6. Distribution of the methylated proteins between **particulate** and cytosolic fractions was found to be similar to that of the small GTP-binding proteins (i.e., a 4:1 ratio). (ABSTRACT TRUNCATED AT 400 WORDS)

L8 ANSWER 14 OF 15 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 89255569 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2722887
TITLE: Post-translational **isoprenylation** of cellular proteins is altered in response to mevalonate availability.
AUTHOR: Repko E M; Maltese W A
CORPORATE SOURCE: Weis Center for Research, Geisinger Clinic, Danville, Pennsylvania 17822.
CONTRACT NUMBER: R01 CA 34569 (NCI)
SOURCE: Journal of biological chemistry, (1989 Jun 15) 264 (17) 9945-52.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198907
ENTRY DATE: Entered STN: 19900306

Last Updated on STN: 19970203

Entered Medline: 19890713

AB **Cells incorporate** isoprenoid products derived from mevalonate (MVA) into several unique proteins. The aim of this study was to delineate the effects of blocking MVA synthesis on the covalent **isoprenylation** of these proteins in murine erythroleukemia cells. Inhibition of protein synthesis with cycloheximide prevented the incorporation of [3H]MVA into proteins, suggesting that **isoprenylation** normally occurs immediately after synthesis of the polypeptides. However, incubation of cells with lovastatin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, for as little as 1 h prior to addition of cycloheximide rendered the **isoprenylation** step insensitive to cycloheximide. Lovastatin had no apparent effect on the stability of the **isoprenylated** proteins, but the development of cycloheximide insensitivity during the lovastatin preincubation was dependent on synthesis of new protein during that period. Addition of 50-200 microM MVA to the culture medium eliminated the effects of preincubation with lovastatin. Preincubation of cells with 25-hydroxycholesterol, which suppresses the synthesis and enhances the degradation of HMG-CoA reductase but is not a competitive enzyme inhibitor, did not induce cycloheximide-insensitivity of the **isoprenylation** reaction. The results suggest that blocking MVA synthesis with lovastatin causes a rapid depletion of isoprenoid groups available for protein modification. Consequently, there is an accumulation of non-**isoprenylated substrate** proteins. Shifts in the ratio of modified vs. unmodified proteins in response to MVA availability may have implications for the changes in cell morphology, cell proliferation and HMG-CoA reductase gene expression that occur when cells are subjected to MVA deprivation.

L8 ANSWER 15 OF 15 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 88087450 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3693410
TITLE: **Isoprenylated** proteins in cultured cells:
subcellular distribution and changes related to altered
morphology and growth arrest induced by mevalonate
deprivation.
AUTHOR: Maltese W A; Sheridan K M
CORPORATE SOURCE: Department of Neurology, College of Physicians & Surgeons
of Columbia University, New York, New York 10032.
CONTRACT NUMBER: RO1 CA 34569 (NCI)
SOURCE: Journal of cellular physiology, (1987 Dec) 133 (3) 471-81.
Journal code: 0050222. ISSN: 0021-9541.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198801
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19970203
Entered Medline: 19880125

AB In the presence of lovastatin (mevinolin), an inhibitor of endogenous mevalonate synthesis, C1300 murine neuroblastoma **cells incorporated** (2-14C)mevalonate into several discrete polypeptides that were separable by SDS-PAGE. The electrophoretic pattern of the labeled proteins did not vary substantially when cells were homogenized with Ca++, Mg++, high concentrations of NaCl or phosphatase inhibitor, or when cells were lysed immediately in trichloroacetic acid. When cells that had been prelabeled with (14C)mevalonate were incubated with lovastatin and simultaneously deprived of exogenous mevalonate, there was

a 50-60% decline in the concentration of protein-bound isoprenoid label within 17 h. In contrast, there was little change in the radioactivity in the sterol, dolichol, or ubiquinone fractions. The time course of the decline in mevalonate-derived label in cellular polypeptides paralleled the onset of neurite outgrowth and preceded the decline of DNA synthesis, suggesting that a decreased intracellular concentration of protein-bound isoprenoid groups may contribute to the well-documented effects of mevalonate deprivation on cell morphology and cell cycling. Fractionation of neuroblastoma cells by differential centrifugation and sucrose density-gradient centrifugation revealed that mevalonate-labeled proteins of 53 kDa, 22-26 kDa, and 17 kDa were concentrated in the cytosol. Proteins migrating at 45 kDa were found in both the soluble and **particulate** fractions, including those enriched in mitochondria and plasma **membrane**. The **isoprenylated** proteins migrating at approximately 66 kDa were localized exclusively in the nuclear fraction. When chromatin was removed from the nuclei by extraction with 2 M NaCl, the 66 kDa **isoprenylated** proteins remained associated with the residual components of the nuclear matrix and lamina. **Isoprenylated** proteins with electrophoretic mobilities similar to those observed in neuroblastoma cells were detected in a variety of established cell lines. However, there was considerable variation among cell lines in the overall efficiency of protein labeling with (¹⁴C) mevalonate and in the prominence and mobilities of specific labeled proteins in the 45-70 kDa range. Comparisons of paired transformed vs. nontransformed fibroblast cell lines suggested that the profile of mevalonate-labeled proteins in a given cell line is not altered by malignant transformation. (ABSTRACT TRUNCATED AT 400 WORDS)

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L7 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:512663 HCAPLUS
DOCUMENT NUMBER: 139:176103
TITLE: Selective enrichment of thiophosphorylated
polypeptides as a tool for the analysis of protein
phosphorylation
AUTHOR(S): Kwon, Sung Won; Kim, Sung Chan; Jaunbergs, Janis;
Falck, John R.; Zhao, Yingming
CORPORATE SOURCE: Department of Biochemistry, University of Texas
Southwestern Medical Center, Dallas, TX, 75390-9038,
USA
SOURCE: Molecular and Cellular Proteomics (2003), 2(4),
242-247
CODEN: MCPOBS; ISSN: 1535-9476
PUBLISHER: American Society for Biochemistry and Molecular
Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A chemoselective alkylation method is described for the isolation and
subsequent identification of thiophosphorylated peptides/proteins. The
method involves thiophosphorylation of proteins using adenosine
5'-O-(thiotriphosphate) (ATPγS) followed by selective in situ
alkylation of the newly thiophosphorylated proteins resulting in a stable
covalent bond. The chemoselective alkylation exploits the relatively high
nucleophilicity at low pH of the sulfur in thiophosphate residues, whereas
the nucleophilicities of phosphates, amines, and other functionality of
amino acids are negligible or significantly suppressed. Modified
alkylation reagents linked to biotin or solid supports (e.g. glass or
Sephadex beads) with or without a photocleavable linker facilitate the
isolation of the thiophosphorylated peptide/proteins. This approach is
demonstrated through the localization of phosphorylation sites on myosin
regulatory light chain. We anticipate that this technique will be useful
for isolation and subsequent identification of newly thiophosphorylated
proteins, produced either in vivo or in vitro, thus facilitating the
dissection of protein phosphorylation networks.

CC 9-5 (Biochemical Methods)

Section cross-reference(s): 6

ST thiophosphorylated polypeptide tool analysis protein phosphorylation

IT Protein motifs

(phosphorylation site; selective enrichment of thiophosphorylated
polypeptides as tool for anal. of protein phosphorylation)

IT Laser ionization mass spectrometry

(photodesorption, matrix-assisted; selective enrichment of
thiophosphorylated polypeptides as tool for anal. of protein
phosphorylation)

IT Laser desorption mass spectrometry

(photoionization, matrix-assisted; selective enrichment of
thiophosphorylated polypeptides as tool for anal. of protein
phosphorylation)

IT Phosphorylation, biological

(protein; selective enrichment of thiophosphorylated polypeptides as
tool for anal. of protein phosphorylation)

IT Time-of-flight mass spectrometry

(selective enrichment of thiophosphorylated polypeptides as tool for
anal. of protein phosphorylation)

IT Proteins

RL: BSU (Biological study, unclassified); BIOL (Biological study)

(selective enrichment of thiophosphorylated polypeptides as tool for anal. of protein phosphorylation)

IT 35094-46-3

RL: ARU (Analytical role, unclassified); ANST (Analytical study)

(selective enrichment of thiophosphorylated polypeptides as tool for anal. of protein phosphorylation)

IT 35094-46-3

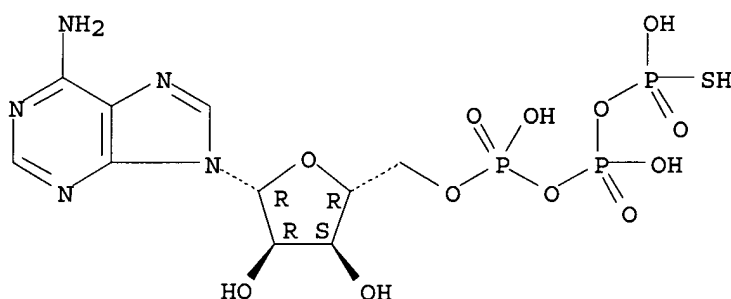
RL: ARU (Analytical role, unclassified); ANST (Analytical study)

(selective enrichment of thiophosphorylated polypeptides as tool for anal. of protein phosphorylation)

RN 35094-46-3 HCAPLUS

CN Adenosine 5'-(trihydrogen diphosphate), P'-anhydride with phosphorothioic acid (9CI) (CA INDEX NAME)

Absolute stereochemistry.



REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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